2979-Pos Board B84
Structure-Based Design of Phospholamban Mutants for Treatment of Heart Failure
Simon J. Gruber, David D. Thomas.
Ca^{2+} cycling through the SR in muscle cells is largely controlled by the Ca-pump (SERCA). SERCA transports Ca^{2+} into the SR, allowing muscles to relax, and is inhibited by phospholamban (PLB) at submicromolar [Ca^{2+}]. PLB inhibition can be relieved by adrenergic stimulation, leading to PLB phosphorylation. Heart Failure (HF), which contributes to 12% of US deaths, can be caused by a variety of genetic or environmental factors, but a common symptom is decreased SERCA activity. We are using EPR and NMR to study the relationships among structure, dynamics, and function of PLB, with the goal of designing LOF-PLB mutants (PLB_M) that can compete with WT-PLB and thus relieve SERCA inhibition. Several studies have shown that a pseudophosphorylated PLB (S16E-PLB) is effective for gene therapy in rodents and sheep, and we are using spectroscopic methods to refine this approach. We have developed a system for examining the function and interactions of SERCA and PLB in HEK cells. Active SERCA is expressed and cells are co-transfected with WT-PLB and/or PLB_M to measure SERCA inhibition in living cells. Effects of PLB/PLB_M on SERCA specific activity (s^{-1}), as well as PLB oligomeric states are characterized in stable cells lines. We can also measure a mutant's ability to compete with WT-PLB by measuring fluorescence resonance energy transfer (FRET) between labeled SERCA and WT-PLB. If PLB_M displaces WT-PLB, less energy is transferred between fluorophores and a decrease in FRET is observed. Preliminary results have led to rAAV-mediated expression of a PLB_M (P21G) in cardiomyocytes of a rat HF model, increasing contractility to near WT levels.

2980-Pos Board B85
Analyzing the Role of NCKX2 in Hippocampal Long Term Potentiation
Yuntian Zhang, Ray W. Turner, Jonathan Lytton.
Na+/Ca2+ exchangers control clearance of cytosolic Ca2+ following a signaling event when Ca2+ is elevated due to their low-affinity/high capacity transport properties. NCKX2 is a member of the family of K+-dependent Na+/Ca2+-exchangers, and is abundant in brain neurons. Targeted knockout of the NCKX2 gene results in a profound loss of long term potentiation and an increase in long term depression at hippocampal Schaffer/CA1 synapses. We examined the expression of a variety of synaptic molecules when NCKX2 is knocked out to disclose the role of NCKX2 in hippocampal plasticity. Co -localization experiments using confocal and electron microscopy were conducted to define the location of NCKX2 in hippocampus. Immunoblot results from whole brain lysates showed that GluR2/3 and NMDA-receptor 1 were reduced in NCKX2 knockout mice. There was no statistical difference in immunoblot for CamKII, PSD95, or GluR1 between wild type and the knockout. In hippocampus phospho-CamKII was upregulated, and small but insignificant changes were found in the expression of other synaptic marker proteins. Co-localization analyses showed minimal overlap of NCKX2 with either the post synaptic marker CamKII or the presynaptic markers synapsin, synaptophysin or VGLUT-1. Immunocytochemistry revealed a decrease in net MAP-2 label in the CA3 region of knockout animals that correlates to a reduction in the number of neuronal profiles and increase in DAPI-labeled nuclei, suggesting neuronal loss and glial cell proliferation. From the above experiments, we can for first time approach the idea of the exact location of NCKX2. Expression change for various synaptic molecules in hippocampus is not obvious, but rather loss of CA3 neurons and the associated Schaeffer collateral projection to CA1, potentially resulting from changes in calcium dynamics when NCKX2 is knocked out.

Protein Ligand Interactions: Receptors & Small Molecules

2981-Pos Board B86
Anesthetic Modulation of Signal Transduction Pathways in the α4β2 nAchR Revealed by the Perturbation-Based Markovian Transmission Model
Lu Tian Liu, Mingzhu Li, David Mowrey, Hsiao-Mei Lu, Jie Liang, Yan Xu, Pei Tang.
The gating mechanism of ligand-gated ion channels and anesthetic modulation of channel gating remain to be determined. In this study, we used the perturbation-based Markovian transmission model to investigate the time-dependent signal propagation in the neuronal nicotinic acetylcholine receptor (α4β2 nAChR) under initial perturbations by agonist binding. Both open- and closed-channel α4β2 nAChR conformations in the presence and absence of the anesthetic halothane, from our previous computational studies, were examined. We found that signal transduction in α4β2 resembles the conformation wave mechanism. In all systems, the perturbation signal flows from the agonist-binding sites up toward the main immunogenic region, then down to the bottom of the beta barrel, followed by the extracellular (EC)/transmembrane (TM) interface, and finally to the transmembrane domain, as measured by the time dependence of the maximum probability flux. The EC domain of the open- and closed-channel α4β2 conformations showed different dynamic responses to the perturbation at the agonist-binding site: many more residues in the EC domain of the open-channel α4β2 nAChR exhibited frequent fluctuation. In the system with halothane, the signal transduction within pre-TM1 (R207), Cys-loop (F137, P138, F139), and TM2-TM3 linker (L271) slowed down in some of the subunits, but speeded up in others. The changes in signal transduction at the EC/TM interface may underlie the effects of anesthetics on the α4β2 nAChR. Supported by NIH (R01GM066358, R01GM056257, R37GM049202).

2982-Pos Board B87
Catharanthine Alkaloids are Noncompetitive Inhibitors of Muscle-Type Nicotinic Acetylcholine Receptors
Ruin Moaddel.
This is an attempt to characterize the binding sites and the mechanisms of action of several catharanthine alkaloids including ibogaine, vincristine, and vinblastine, on muscle-type nicotinic acetylcholine receptors (AChRs), by comparing their pharmacological properties with that for the well characterized high-affinity noncompetitive antagonist (NCA) phencyclidine (PCP). In this regard, structural and functional approaches were used including radioligand equilibrium and competition binding assays using [^{3}H]ibogaine and the analog of PCP, [piperidyl-3, 4-^{3}H(*N*)]-*N*-(1-(2 thienyl)cyclohexyl)-3,4-piperidine ([^{3}H]TCP), Ca^{2+} influx determinations, thermodynamic and kinetic measurements using column-immobilized *Torpedo* AChRs, and molecular docking and dynamics studies The results established that: **(a)** the alkaloidsinhibit (±)-epibatidine-induced Ca^{2+} influx in embryonic muscle AChRs with the following potencies (in μM): ibogaine (17 ± 3) > vinblastine (20 ± 5) > vincristine (25 ± 4), that are slightly higher than that for PCP (31 ± 2), **(b)** the alkaloids inhibit [^{3}H]TCP binding, and ibogaine and PCP inhibit [^{3}H]ibogaine binding, to the desensitized *Torpedo* AChR with higher affinity compared to the resting AChR, **(c)** ibogaine binds to the *Torpedo* AChR by an entropy-driven process, and **(d)** ibogaine interacts with a binding domain located between the serine (position 6') and valine (position 13') rings, by a network of van der Waals and polar interactions. Collectively our data indicate that catharanthine alkaloids block agonist-activated ion channels by interacting with a binding domain that is shared with PCP located between the serine and valine rings. This supports the view that the catharanthine moiety, which is shared by ibogaine and vinca alkaloids, is a minimum structural requirement for the interaction of these molecules with the ion channel. In addition, ibogaine and vinca alkaloids may induce and maintain the desensitized state by a mechanism where their dissociation rates are decreased.

2983-Pos Board B88
A Combined Experimental and Simulation Approach to Develop Selective High-Affinity Small-Molecule Inhibitors of Cannabinoid Receptors CB1/CB2
Irene Meliciani, Nicole Volz, Viktor Rempel, Sonja Hinz, Tadeusz Karcz, Christa E. Müller, Stefan Bräse, Wolfgang Wenzel.
The cannabinoid receptors belong to the membrane-bound G protein-coupled receptor superfamily and are predominantly coupled via Gi/o proteins. The CB1 receptor subtype is known to play an essential role in analgesia, memory-impairment, spasmolysis and regulation of appetite. Selective agonists for CB2 receptors show antiinflammatory and analgesic properties in animal models [1]. Cannabinoid receptors are activated by three major groups of ligands, mammalian endocannabinoids, plant and synthetic cannabinoids (e.g. THC from the plant Cannabis sativa). Here we investigated the interactions between the cannabinoid receptors type 1 and 2 (CB1/CB2) with a novel set of coumarin derivatives.
Rational drug design for many GPCRs is complicated by the lack of receptor crystal structures. To aid interpretation of the experiments we have therefore constructed a model for the CB1 and CB2 receptors based on homology to bovine rhodopsin (pdb-code 1U19) and performed ligand binding simulations for a family of 39 coumarin derivatives applying an all-atom docking protocol using Flexscreen [2].